# Figure 1A

Mutagenize Rgene by error-prone PCR

Ligate Rgene\* to expression vector

Transform into ER2502 [pMgene]

Select survivors at 25°C-42°C on LB agar + Amp, Cam, IPTG

Make a plasmid library of pRgene\* and pMgene

Eliminate pMgene by digestion, but leave pRgene\* intact

Transform ER1992 dinD::lacZ indicator strain with pRgene\* library

Select individual dark blue colonies at 30°C-42°C on LB agar + X-gal, Amp (No IPTG)

Prepare plasmid DNA from individual dark blue colonies

Transform pRgene\* clones into ER1992 [pMgene]

Select white colonies at 30°C-42°C on LB agar + X-gal, Amp, Cam

The white colonies may contain Rgene\* variants with desired specificity

Screen white colonies

## Figure 1B

## Genetic Selection of BstYI Variants with 5'-AGATCT-3' specificity

Mutagenize bstYIR gene by error-prone PCR

Ligate bstYIR\* to pAGR3 (Ptac)

Transform into ER2502 [pSYX-BglIIM]

Select survivors at 37°C on LB agar + Amp, Tet, 0.3 mM IPTG

Make a plasmid library of pAGR3-BstYIR\* and pSYX-BglIIM

Eliminate pSYX-BglIIM by digestion, but leave pAGR3-BstYIR\* intact

Transform ER1992 dinD::lacZ indicator strain with pAGR3-BstYIR\* library

Select individual dark blue colonies at 37°C on LB agar + X-gal, Amp (No IPTG)

Prepare plasmid DNA from individual dark blue colonies

Transform pAGR3-BstYIR\* clones into ER1992 [pSYX-BglIIM]

Select white colonies at 37°C on LB agar + X-gal, Amp, Tet

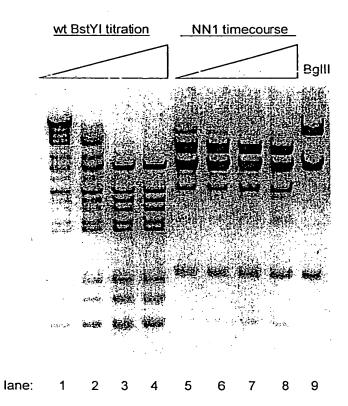
Screen white colonies

The white colonies may contain BstYI\* mutants with BglII specificity

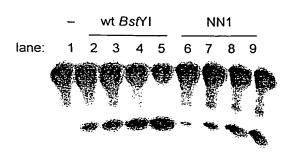
# FIGURE 2

																			CTTG +
CC	ACA	TAT	Τ̈́TG	GGA	AGA	AA1	TCA	AGA	TAA	TAT	TGT	TIC	TAT	TGA	CGC	AGA	AGC	TTG	L TAGA
																	Α		+ R
																			TTTA +
																	V		L .CTAT
			-+-			+				+		- <b>-</b> -	-+-			1	- <b>-</b> -	- <b></b>	+
																	T CAGA		Y ACAA
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AA	.GA.A	AGT	GAT	'TGA	AGC	CGC	AGG	AAA	AGA	AGC	'ATI	'AAA'	GTC	TT	<b>ATA</b>	ATC	AAA	CGGA	.TTTI
																			F
																			TGAC
v	K	D	R	v	A	I	E	V	Q	F	G	K	Y	s	F	V	Α	Y	D
																			AAAT? 
ТТ	GCC	:AAT	GAA	∆GG₽	T'A	YTA'	AAA	AAG	LAA!	GTO	TTC	CGG	GAA:	rca(	GTT	ATT		AAG	STGAZ
L	P	M	K	E	L	s	K	E	M	S	s	G	I	s	Y	Y	E	G	E rcgg(
																	+ L		<b>-</b> -
ΓA	TGC	CCC	TT	λA		Q	G	ĸ	G	V	r	A	V	r	J	V		1.	G
			*		<u>.</u>														

Figure 3



### FIGURE 4A



2 4 6 10 5 10 20 30

5'.....AGATCT.....3' 3'.....TCTAGA.....5'

## FIGURE 4B



5'.....GGATCC.....3' 3'.....CCTAGG.....5'

time: - 2 4 6 10 5 10 20 30

### FIGURE 4C



5'.....AGATCC.....3' 3'.....TCTAGG.....5'

time: - 2 4 6 10 5 10 20 30

Figure 5

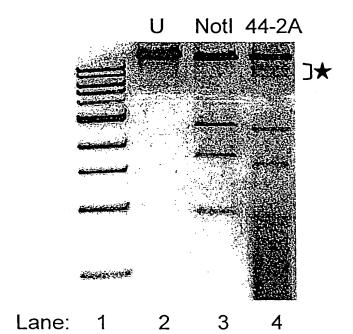


Figure 6

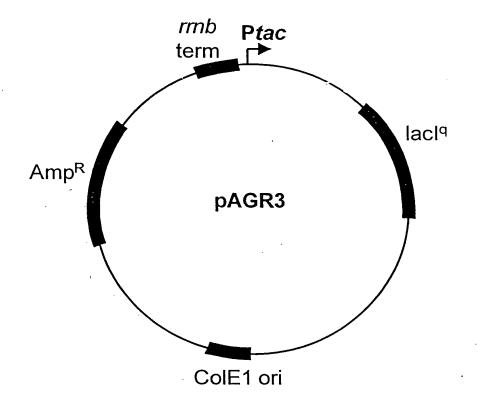


Figure 7

